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Construction and Screening of a 2-Aminoimidazole Library Identifies a Small Molecule Capable of Inhibiting and Dispersing Bacterial Biofilms across Order, Class, and Phylum**

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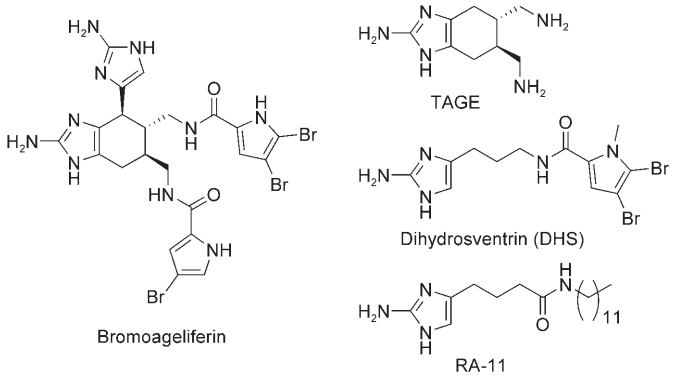
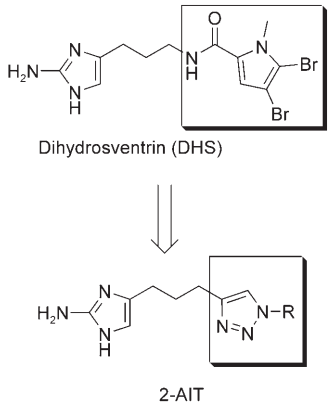
Bacterial biofilms are defined as a surface-attached community of bacteria that are surrounded by a protective extracellular matrix.[1] Within the biofilm state, bacteria display differential gene expression and are at least 1000-fold more resistant to antibiotic treatment.[2] Biofilms account for more than 80% of all bacterial infections; they drive persistent infection of indwelling medical devices, and are responsible for the mortality and morbidity of almost all cystic fibrosis (CF) patients.[3–6]

Given the biomedical prominence of biofilms, there have been significant efforts to discover small molecules that modulate biofilm development.[1] We have shown that simple derivatives of the marine natural product bromoageliferin will both inhibit and disperse bacterial biofilms (Scheme 1).[7–11] Recently, we demonstrated that dihydrosventrin (DHS) inhibits and disperses *Pseudomonas aeruginosa* (multiple strains), *Acinetobacter baumannii*, and *Bordetella bronchiseptica* biofilms,[7] making it the first small molecule reported to inhibit and disperse biofilms across bacterial order and class through a nonbactericidal mechanism.

We have begun to investigate whether modifications to the core DHS structure will lead to derivatives with enhanced anti-biofilm activities.[8] One of the first structural variations we have studied is replacement of the pyrrole subunit with a triazole subunit (Scheme 2). Herein we detail the development of the synthetic protocols necessary to access 2-aminoimidazole/triazole conjugates (2-AITs), the application of these methods to the synthesis of a focused 2-AIT library, and the discovery of small molecules that inhibit and disperse bacterial biofilms across order, class, and phylum.



Scheme 1. Bromoageliferin, structural inspiration for the synthesis of analogues. TAGE, DHS, and RA-11 are analogues that inhibit and disperse bacterial biofilms.



Scheme 2. Design of a 2-AIT.

Given that there is a paucity of reactions that have been reported to be compatible with 2-aminoimidazoles, we deemed the Cu^I-catalyzed [3+2] alkyne/azide cycloaddition (click reaction)[12–14] as a promising method to generate 2-AITs given the broad substrate range displayed by this reaction. To test the applicability of the reaction, we synthesized the alkynyl-substituted 2-aminoimidazole (2-AI, **1**) and tested its ability to participate in a Cu^I-catalyzed [3+2] cycloaddition with benzyl azide.

Amino acid **2**[15] was subjected to a small-scale Akabori reduction,[16] which, followed by condensation with cyanamide[17] delivered the target alkyne **1** in 88% yield (Scheme 3). With **1** in hand, we explored various conditions to elicit the Cu-catalyzed [3+2] cycloaddition between **1** and benzyl azide (Table 1). In THF with Cu^I only starting material

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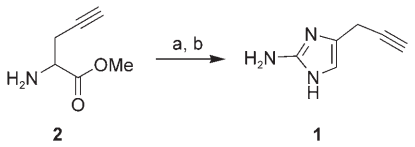
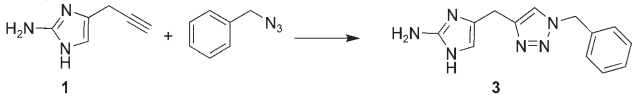
Supporting information for this article (compound synthesis, compound characterization, initial library screening, bacterial growth curves, bacterial colony counts, and dose–response curves) is available on the WWW under http://www.angewandte.org or from the author.



Scheme 3. Synthesis of alkynyl-substituted 2-aminoimidazole **1**. a) 5% Na/Hg, H_2O, pH 1.5–2.0, 0–5 °C. b) Cyanamide, H_2O, pH 4.3, 95 °C.

Table 1: Results of the Cu-catalyzed [3+2] cycloaddition between **1** and benzyl azide under various conditions.



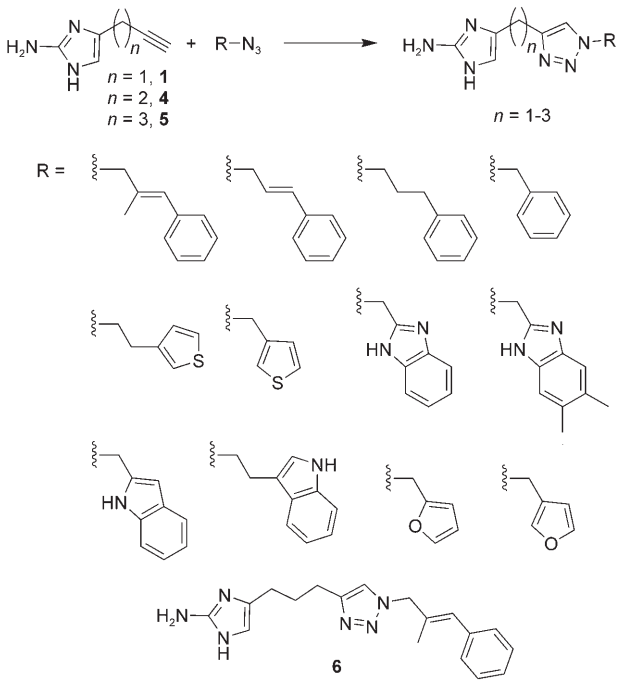
Scale [mg]	Cu^I source[a]	Solvent	Base[b]	*T* [°C]	Yield [%]
20	CuI	THF	DIEA	RT	–
20	CuI	THF	DIEA	40	–
20	$CuSO_4$/NaAsc	EtOH/H_2O (1:1)	–	RT	–
20	$CuSO_4$/NaAsc	EtOH/H_2O (1:1)	–	40	86
100	$CuSO_4$/NaAsc	EtOH/H_2O (1:1)	–	40	decomposition
100	$CuSO_4$/NaAsc	*t*BuOH/H_2O/CH_2Cl_2 (1:1:1)	–	RT	93

[a] NaAsc = sodium ascorbate. [b] DIEA = diisopropylethylamine.

was returned. We then switched to using $CuSO_4$ and sodium ascorbate in a 1:1 solvent mixture of EtOH/H_2O. Again, no reaction was noted. However, when the reaction mixture was heated to 40 °C the desired 2-AIT **3** was obtained in 86% yield. Unfortunately, when the reaction was scaled up, a significant amount of decomposition occurred. Room-temperature click reactions have been described for a 1:1:1 solvent mixture of *t*BuOH/H_2O/CH_2Cl_2.[18] With these reaction conditions we observed conversion to **3** in 93% yield.

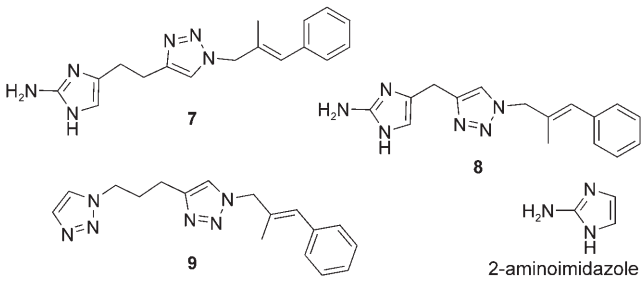
Next we synthesized the 2-AI alkynes **4** and **5** (see Scheme 4) with longer methylene spacers between alkyne and 2-AI units analogous to **1** and used all three of them for click reactions with the 12 azides depicted in Scheme 4 to yield an initial 2-AIT library. Each compound was characterized (^{1}H NMR, ^{13}C NMR, HRMS) prior to screening (Supporting Information).

Each 2-AIT was initially screened at 300 μM for its ability to inhibit *P. aeruginosa* PAO1 and PA14 biofilms, *A. baumannii* biofilms and *B. bronchiseptica* RB50 biofilms using a crystal-violet reporter assay.[19] From this screen, 2-AIT **6** was identified as the most promising; it was most active against *A. baumannii*, with an observed IC_{50} of 12 μM. This is an order-of-magnitude increase in activity when compared to DHS.[7] Follow-up growth curves and colony counts indicated that **6** had no effect on planktonic growth (Supporting Information), indicating that the inhibition of biofilm development was not due to microbicidal activity. Compounds **6** also dispersed preformed *A. baumannii* biofilms with an EC_{50} of 400 μM.



Scheme 4. Construction of the initial 2-AIT library.

The effect of the tether length on activity was addressed by determining the IC_{50} of *A. baumannii* biofilm inhibition for **7** and **8** using dose–response studies. Compound **7** showed an

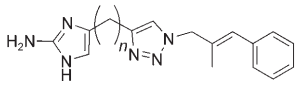


IC_{50} of 220 μM, and **8** did not inhibit *A. baumannii* biofilm development in a significant fashion (< 50%) at 800 μM (highest concentration tested). Thus, increasing tether length appears to correlate with increasing activity. In addition, we tested the necessity of the 2-AI subunit by synthesizing compound **9**—by alkylating commercially available 1-H-1,2,3-triazole with 5-iodopent-1-yne and subjecting the resulting alkynyl-substituted triazole to a click reaction—and assaying for its ability to inhibit *A. baumannii* biofilm development. This compound revealed minimal activity (<30% inhibition) at the highest concentration studied (800 μM). 2-Aminomidazole was also screened and found to be devoid of activity up to 800 μM (highest concentration tested).

As indicated above, as the number of methylene units between 2-AI and triazole unit are increased (**6**–**8**), the anti-biofilm activity against *A. baumannii* also increases. Therefore, we synthesized 2-AITs **10**–**12** where we systematically

extended the methylene linker to investigate if additional methylene unit would deliver a 2-AIT with even greater biological activity than **6** (Table 2). Compound **10** showed an IC_{50} value of 2.8 μM against *A. baumannii* as well as IC_{50} values of 15, 4.0, and 23 μM against PAO1, PA14, and RB50, respectively. We also tested its ability to inhibit *Staphylococcus aureus* biofilm development and found an IC_{50} value of 7.0 μM. Colony counts and growth curves of each bacterial strain grown in the presence of **10** revealed that its activity was not due to bactericidal activity, which, to the best of our knowledge, is the first example of a nonbactericidal small molecule that will inhibit biofilm development across order, class, and phylum.[1] Increasing the methylene spacer to 5 carbon atoms (**11**) again led to an increase in activity (see Table 2). Further addition of a methylene group (**12**) did not lead to an increase in activity (see Table 2). Follow-up colony count and growth curve analysis revealed that inhibition of biofilm development for both **11** and **12** was not due to microbicidal activity.

Table 2: Effect of chain length on the activity of 2-AITs against several biofilms.[a]



n	*A. baumannii*	PAO1	PA14	RB50	*S. aureus*
4 (**10**)	2.8	15	4.0	23	7.0
5 (**11**)	0.98	5.6	0.53	9.5	0.81
6 (**12**)	6.8	2.7	22	70	4.6

[a] IC_{50} values are given in μM.

Finally, we have tested for the ability of a single administration of 2-AITs **10**–**12** to disperse preformed bacterial biofilms. The summary of these experiments is outlined in Table 3. As can be seen, each compound was able to disperse the preformed biofilm, regardless of bacterial order, class, or phylum.

Table 3: Efficiency of compounds **10**–**12** in dispersing preformed biofilms.[a]

Compound	*A. baumannii*	PAO1	PA14	RB50	*S. aureus*
10	210	81	35	59	16
11	120	11	22	55	2.6
12	36	51	48	75	37

[a] EC_{50} values are given in μM.

In conclusion, we have developed a synthetic approach to 2-aminoimidazole/triazole conjugates that is underpinned by Cu^{I}-catalyzed [3+2] alkyne–azide cycloaddition. Using this chemistry we have assembled a focused library of 2-AITs and, with an initial hit from this library as lead, derived compounds that are able to inhibit and disperse bacterial biofilms across order, class, and phylum. Mechanistic studies are currently underway to determine how 2-AITs **10**–**12** inhibit and disperse biofilms. Furthermore, given the promising anti-biofilm activity displayed by these and other 2-AI derivatives,[7–11] we are continuing to develop methodology to access further functionalized libraries based upon the 2-AI core motif. These studies will be disclosed in due course.

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